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MINIREVIEW

Regulation of Sugar Utilization in *Saccharomyces* Species

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INTRODUCTION

Yeasts of the genus *Saccharomyces* are able to utilize a wide variety of sugars as sources of carbon and energy (for reviews, see references 2 and 26). Sugar utilization is governed by both genetic capability and regulatory mechanisms. In this review, I will first briefly consider the genetic variation in ability to utilize sugars that is found among *Saccharomyces* strains. I will then discuss the global regulation of sugar utilization by glucose repression. Finally, I will discuss mechanisms for the regulation of sucrose, galactose, melibiose, and maltose metabolism by glucose repression and by induction.

GENETIC VARIABILITY: FERMENTATION GENE FAMILIES

Closely related *Saccharomyces* strains differ greatly in their ability to utilize sugars. Fermentation of disaccharides and oligosaccharides is controlled by dispersed repeated families of genes, such as the *SUC* (sucrose), *MAL* (maltose), *MEL* (melibiose), and *MGL* (α-methylglucoside) gene families. Each family includes multiple, unlinked, functionally equivalent loci that control fermentation ability; for example, the *SUC* family includes six unlinked *SUC* genes, each a structural gene for invertase. An unusual feature of these gene families is that closely related *Saccharomyces* strains carry different active members of each family, and some strains lack functional genes. As a result, *Saccharomyces* strains differ in their ability to ferment sugars.

Studies of the molecular basis for the variability in *SUC* and *MAL* genotype indicate that the fermentation genes are present at different chromosomal locations in different genomes (reviewed in reference 6). For example, one strain may have a *SUC* gene at the *SUC1* locus, whereas another strain may have no *SUC* gene sequences at that locus (7). Analysis of the *SUC* family indicates that most *SUC* genes reside near chromosome telomeres and that dispersal of *SUC* genes to different chromosomes occurred by rearrangements of telomeres (8). The exception is the *SUC2* locus, which is not telomeric and probably always carries *SUC* gene sequences, although not always a functional allele (7). The overall organization of the *MAL* gene family appears to be similar to that of the *SUC* gene family, although each *MAL* locus is a complex locus including several genes involved in maltose utilization (14, 44, 47). The genotypic variability associated with these fermentation gene families is an unusual feature of the *Saccharomyces* genome. These dispersed gene families may offer the organism versatility in adaptation to different environments.

GLUCOSE REPRESSION

In a yeast strain with the genetic capability to utilize a particular sugar, the utilization of that sugar is controlled by glucose repression or carbon catabolite repression. Glucose repression is a global regulatory system governing the response of cells to the availability of glucose, and it affects expression of a multitude of genes, including sugar fermentation genes. Yeasts preferentially utilize hexoses such as glucose and fructose that enter the glycolytic pathway directly; thus, sugars such as sucrose and galactose are not metabolized in the presence of glucose. Glucose repression regulates expression of fermentation genes at the transcriptional level. Glucose also affects transport of sugars into the cell and thereby affects the function of regulatory mechanisms for induction.

Studies in several laboratories have shown that glucose repression is a complex regulatory system, and more than a dozen putative regulatory genes have been identified by genetic analysis (for reviews, see references 21 and 27). It is perhaps not surprising that many genes are involved, as the regulatory mechanisms must include sensory and signaling mechanisms for monitoring glucose availability and regulatory proteins that effect changes in expression of a multitude of genes. Although our picture of the regulatory circuitry is still incomplete, the regulatory system of *Saccharomyces cerevisiae* appears to be different from that of *E. coli*. The molecular signal for glucose deprivation in *E. coli* is cyclic AMP, which activates the catabolite activator protein, which in turn activates transcription of catabolite-repressible operons, such as *lac*. In contrast, genetic and biochemical evidence indicates that cyclic AMP is not a direct effector for glucose repression in *S. cerevisiae* (25, 41, 42). The information available on the molecular mechanisms of glucose repression in *S. cerevisiae* also supports the view that the regulatory system of *S. cerevisiae* is different from that of *E. coli*. Biochemical functions are known for two genes that affect global glucose repression, *HXK2* and *SNF1*. *HXK2* is the structural gene for the hexokinase PII (B) isozyme. Entian and his colleagues have isolated *hxk2* mutations that do not affect the catalytic function but cause constitutivity for glucose-repressible enzymes, suggesting a regulatory function (20, 22, 23). My group has recently shown that *SNF1*, a gene required for release from glucose repression, encodes a protein kinase (11). This finding suggests that protein phosphorylation has a critical role in the mechanism for glucose repression in *S. cerevisiae*.

Genetic analysis of glucose repression has generally involved isolation of mutants that affect regulation of a particular "reporter" gene. A variety of schemes has been employed to isolate mutants with defects in glucose repression or derepression of different reporter genes. Many mutations

TABLE 1. Genes affecting glucose repression of *SUC2*

Gene[a]	Effect of mutation on *SUC2* expression	Gene product
SNF1 = *CCR1* = *CAT1*	Defective derepression	Protein kinase
SNF4	Defective derepression	
SNF2	Defective derepression	
SNF5	Defective derepression	
SNF6	Defective derepression	
SNF3	Defective derepression and defective repression[b]	Glucose transporter
HXK2 = *HEX1*	Defective repression	Hexokinase PII
REG1 = *HEX2*?	Defective repression	
CID1	Defective repression	
SSN6 = *CYC8*	Defective repression	
TUP1 = *CYC9* = *UMR7* = *FLK1*	Defective repression	

[a] Genes are grouped according to epistasis relationships (see text).
[b] Phenotypes of *snf3* missense mutations; *snf3* null mutations do not affect *SUC2* expression.

have been found to cause defects in regulation of a spectrum of glucose-repressible genes, but not of all glucose-repressible genes. Thus, glucose repression is not effected only by global mechanisms; rather, some aspects of the regulatory system are specific to subsets of glucose-repressible genes. Mutants that have been obtained by using a variety of procedures have been recently reviewed by Gancedo and Gancedo (27) and by Entian (21). This review will consider only studies relating to glucose repression of the particular sugar utilization systems described below.

SUCROSE UTILIZATION

Utilization of sucrose or the trisaccharide raffinose requires expression of one of the *SUC* genes (*SUC1* through *SUC5*, *SUC7*) encoding invertase. The regulation of sucrose utilization is in principle less complex than that of other sugars because regulation is solely by glucose repression. There is no induction of *SUC* gene expression in response to availability of the substrates sucrose and raffinose. Nonetheless, regulation of *SUC* expression has proved to be a complex process requiring many genes (Table 1). Mutants with defects in either derepression or repression of *SUC2* have been isolated, and all show defects in regulation of other glucose-repressible genes as well.

My group has shown that regulation of *SUC2* occurs at the transcriptional level and is mediated by an upstream regulatory region (57). We isolated recessive mutations in six genes, *SNF1* through *SNF6*, that caused defects in derepression of *SUC2* and other glucose-repressible genes (9, 48). *SNF1* is the same gene as *CCR1* and *CAT1*, which were independently found to be essential for derepression of several glucose-repressible genes (15, 21). We have shown that the *SNF1* gene encodes a protein kinase (11). Analysis of the genetic interactions of *snf* mutations with extragenic suppressors suggested that *SNF4* is functionally related to *SNF1*, perhaps as a regulator or target of the protein kinase, and that *SNF2*, *SNF5*, and *SNF6* are related to one another (48, 50). Further study of *SNF2*, *SNF5*, and *SNF6* suggested that these genes are required for high-level expression of *SUC2* and other genes but may not play regulatory roles in glucose repression (1). The *SNF3* gene encodes a protein that is homologous to a human glucose transporter (J. Celenza, L. Marshall-Carlson, and M. Carlson, manuscript in preparation) and is required for high-affinity glucose transport (3). Disruption of *SNF3* showed that *SNF3* is not essential for regulation by glucose repression, but the aberrant regulation of *SUC2* expression observed in *snf3* missense mutants leaves open the possibility of a regulatory role for the *SNF3*-encoded protein (51).

Mutations causing constitutive (glucose-insensitive) synthesis of secreted invertase have been isolated in several laboratories. Zimmermann and Scheel isolated the *hex1*, *hex2*, and *cat80* mutations, which cause constitutivity for invertase and also for maltase and malate dehydrogenase (24, 70). *HEX1* is the same gene (20) as *HXK2*, the structural gene for hexokinase PII, which has been postulated to play both catalytic and regulatory roles in carbon source utilization (22, 23). Genetic mapping suggests that *hex2* (22) is allelic to *reg1* (43), which causes glucose-insensitive expression of galactokinase and invertase. In a selection for constitutive mutations in the S288C genetic background, my group recovered additional *hxk2* and *reg1* alleles and also mutations at a new locus designated *cid1* (49). Mutations at a locus called variously *tup1*, *umr7*, *flk1*, and *cyc9* cause constitutivity for invertase, maltase, and other glucose-repressible enzymes and also cause pleiotropic defects that are not obviously related to glucose repression (e.g., clumpiness, mating and sporulation defects, and utilization of exogenous deoxythymidine monophosphate) (38, 56, 58, 60, 65, 67). Constitutive mutations at another locus, *ssn6* or *cyc8*, cause a similar spectrum of pleiotropies (10, 56, 65). Our *ssn6* alleles were isolated as suppressors of the sucrose-nonfermenting phenotype caused by *snf1*, and *ssn6* was found to cause high-level constitutive invertase expression in both *snf1* and wild-type (*SNF1*) genetic backgrounds (10).

To assess the roles of these various genes in the regulatory circuits for glucose repression, we analyzed the epistasis relationships between the constitutive mutations and *snf* mutations (48, 49). These studies suggested that the constitutive mutations fall into two classes, one comprising *cid1*, *reg1*, and *hxk2* and the other comprising *ssn6* and *tup1*. The *snf* mutations (except for *snf3*) were epistatic to *cid1*, *reg1*, and *hxk2*, suggesting that *CID1*, *REG1*, and *HXK2* function at early steps in the regulatory circuitry; perhaps these genes perform sensory or signaling functions that enable the cell to evaluate the availability of glucose in the environment. In contrast, *ssn6* and *tup1* suppressed the invertase derepression defect of *snf1*; this finding suggests that the negative regulatory effects of *SSN6* and *TUP1* on gene expression are fairly direct.

GALACTOSE AND MELIBIOSE UTILIZATION

The utilization of galactose requires the Leloir pathway enzymes encoded by the clustered *GAL1*, *GAL7*, and *GAL10* genes and the galactose permease encoded by *GAL2*. The expression of these genes is induced by galactose and repressed by glucose. Utilization of melibiose requires also α-galactosidase, encoded by *MEL1*, which is similarly regulated. Induction is controlled by at least three regulatory genes, *GAL4*, *GAL80*, and *GAL3*. Oshima has reviewed (54) the early studies of this regulatory system and has provided complete references. I will here simply summarize the conclusions of Oshima and then consider more recent work in greater detail. Oshima documents evidence that *GAL4* encodes a positive regulatory protein required for induction

of transcription of the galactose catabolic pathway genes and that *GAL80* encodes a negative regulator that interferes with *GAL4* function in the absence of inducer. The evidence suggests that *GAL4* and *GAL80* are constitutively expressed and that the two proteins interact. In the model favored by Oshima, the *GAL4* and *GAL80*-encoded proteins form a complex, and inducer dissociates the complex (or perhaps inhibits the negative function of the *GAL80* protein), thus allowing transcriptional activation by the *GAL4* protein.

Recent molecular analysis has provided further insight into these regulatory functions. The positive regulatory gene *GAL4* was cloned in several laboratories (31, 33, 36) and was shown to encode a 99-kilodalton protein (37). Increased *GAL4* gene dosage resulted in increased expression of *MEL1* and *GAL* cluster genes under noninducing/nonrepressing and glucose-repressing conditions; these results suggest that a molar excess of *GAL4* protein over the *GAL80* repressor allows transcriptional activation in the absence of inducer (31, 33). This interpretation was supported by evidence that a concomitant increase in *GAL80* gene dosage suppressed the *GAL4* gene dosage effect (31).

Recent biochemical evidence has shown that the *GAL4* product binds to specific sites upstream of the target genes and activates transcription. Giniger et al. (28) showed that the *GAL4* protein binds to four related 17-base-pair sites in the upstream activating sequence (UAS_G) between the divergently transcribed *GAL1* and *GAL10* genes (30, 32, 66, 68). A synthetic 17-base-pair sequence close to the consensus sequence of these binding sites conferred *GAL4*-mediated galactose inducibility to yeast promoters (28). Bram and Kornberg also detected a *GAL4*-dependent binding of a protein to specific sites upstream of *GAL* cluster genes (4). Regions of the *GAL4* protein that are required for nuclear localization, DNA binding, transcriptional activation, and interaction with the *GAL80* negative regulator have been identified (5, 34, 35, 39, 59). Studies from the Ptashne laboratory have provided evidence that DNA binding and transcriptional activation are separable functions, suggesting that the *GAL4* protein bound to the UAS activates transcription by contact with other DNA-bound proteins (5, 35, 39).

The *GAL80* gene has also been cloned (53, 63, 69), and sequence analysis predicts that it encodes a 48-kilodalton protein (52). Gene dosage and gene disruption studies confirmed that the *GAL80* protein is a negative regulator of *MEL1* and *GAL* cluster gene expression (53, 63, 69). Analysis of *gal80* deletion mutants showed that the *GAL80* protein is required only for repression in the absence of inducer and is not necessary for expression of the structural genes (63, 69). As expected, deletion of *GAL80* did not bypass the need for *GAL4* function (63). Also, glucose repression was substantially intact in the absence of *GAL80* function, indicating that the *GAL80* protein does not mediate major effects (63, 69).

These studies indicate that regulation depends on a dosage-dependent functional interaction between the *GAL4*- and *GAL80*-encoded proteins. The mechanism of this interaction is not yet clear. One possibility is still the model favored by Oshima (54), i.e., that the two proteins form a complex that is dissociated in the presence of inducer. Giniger et al. reported preliminary evidence that the *GAL80* protein does not prevent the binding of *GAL4* protein to DNA, suggesting that the *GAL80* protein inhibits *GAL4*-mediated transcriptional activation by modifying the interaction of *GAL4* protein with UAS_G (28); these findings are not inconsistent with the idea that the regulatory mechanism involves dissociation of the complex. Another possibility suggested by several authors, on the basis of indirect evidence, is that the *GAL80* protein may interact with regulatory sequences at the target genes to interfere with *GAL4* function (31, 33, 53).

The third major regulatory gene is *GAL3*. The *GAL3* gene is necessary for the rapid induction of the galactose catabolic enzymes in response to the presence of galactose. A mutation at *GAL3* results in a delay of several days. Genetic evidence (reviewed in reference 54) and recent molecular analysis of the *GAL3* gene (64) indicate that *GAL3* functions in induction at an earlier step than *GAL4* does and that a function provided by either *GAL3* or the *GAL1-10-7* cluster is required for maintenance of the induced state. The currently favored model is that *GAL3* functions in synthesis of the inducer or coinducer.

Studies in several laboratories have provided insight regarding the mechanisms by which glucose repression specifically affects expression of the *GAL* genes. Genetic studies led Matsumoto et al. (43) to propose that three independent regulatory circuits contribute to glucose repression of the *GAL* genes. One of these circuits involves the *REG1* gene and has global effects on many glucose-repressible genes. The other two circuits are specific to galactose-regulated genes. One of the specific circuits involves two genes, designated *GAL82* and *GAL83* (40, 43), and the other is the *GAL4/GAL80* circuit. Matsumoto et al. suggest that glucose affects the *GAL4/GAL80* circuit by affecting intracellular levels of galactose through inhibition of galactose uptake (43); however, Yocum and Johnston have argued that if inducer exclusion plays a role in glucose repression, it must occur at the level of *GAL2* expression, not at the level of galactose permease function (69). Two lines of evidence point to a direct role of the *GAL4* gene product in mediating glucose repression. First, Giniger et al. obtained biochemical evidence that one mechanism of glucose repression involves the inhibition of binding of the *GAL4* protein to UAS_G, which was observed in both the presence and absence of the *GAL80* protein (28). Second, recent studies of *GAL4* function in another yeast, *Kluyveromyces lactis*, have also suggested a role in glucose repression. Introduction of *GAL4* into a *K. lactis* strain with a mutation in *LAC9*, a positive regulatory gene for the lactose/galactose regulon, not only restored activation of the regulon but also caused a dramatic increase in sensitivity to glucose repression (55).

Two additional lines of evidence bear on the problem of glucose repression. Experiments reported by Struhl suggest that UAS_G mediates glucose repression by a negative control mechanism that can act at a distance on a heterologous, non-glucose-repressible promoter (61). Also, West et al. constructed deletions in UAS_G that reduced the repression caused by glucose, although this effect could have resulted from the decreased distance between UAS_G and the TATA box rather than from removal of specific sequences (66).

MALTOSE UTILIZATION

Maltose utilization requires a maltose transport system and maltase. These functions are induced by maltose and repressed by glucose. The genes required for maltose utilization are found at the *MAL* loci (*MAL1* through *MAL4*, *MAL6*). Physical and genetic analyses of *MAL* loci have shown that each active locus includes three genes that most probably encode maltase, a maltose transport protein, and a positive regulatory protein (16–18, 29, 45, 46, 62). Many *Saccharomyces* strains carry "cryptic" *MAL* loci that carry functional copies of some, but not all, of these genes (14, 44, 47).

Expression of the maltase and maltose permease genes is regulated at the RNA level, and the positive regulatory function present at *MAL* loci is required in *trans* for induction of these RNAs (12, 16, 46). Induction of the RNA encoding maltase also depends on the functioning of the maltose transport system (12, 16). Sequence analysis of the regulatory gene from the *MAL6* locus (the *MAL63* gene) revealed a cysteine-lysine-arginine-rich sequence that could form a metal-binding finger, thus raising the possibility that the positive regulatory gene product functions by binding to DNA (J. Kim and C. Michels, personal communication). The regulatory function may be involved in glucose repression as well as maltose induction; a dominant mutation in the regulatory gene at the *MAL4* locus (*MAL43*, which is homologous to *MAL63*) causes both constitutive (maltose-independent) and glucose-repression-insensitive expression of the maltose fermentation genes (13). Recent studies have identified a second *trans*-acting regulatory gene present at the *MAL6* locus that can be activated by mutation (19). Mutations in this gene, called *MAL64*, cause constitutive expression of maltase and maltose permease structural genes. It is not yet known whether any other *MAL* loci carry genes homologous to *MAL64*.

CONCLUDING REMARKS

The regulation of sugar utilization by glucose repression and specific induction systems is clearly complex. Nonetheless, enormous progress has been made in recent years. Genetic analysis has proved extremely useful in providing the framework for a coherent picture of these regulatory circuits, and the application of powerful biochemical and molecular genetic methods has already yielded considerable understanding of molecular mechanisms. Thus, the prospects for understanding these regulatory systems in molecular detail are promising. These systems should provide useful paradigms for studies of the eucaryotic regulatory response to environmental change.

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